

CLAIMS:

1. A method for detecting and quantitating DNA-activated protein kinase (DNA-PK) activity in a biological sample, comprising the steps of:
 - (a) forming a reaction mixture by contacting a biological sample with a detectably-labeled phosphate donor and a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor in the reaction mixture;

(b) incubating said reaction mixture for a time and at a temperature to allow the transfer of phosphate from said phosphate donor to said synthetic peptide substrate;

20 (c) stopping the transfer of phosphate from said phosphate donor to said synthetic peptide substrate;

(d) determining the amount of phosphate transferred from said phosphate donor to said synthetic peptide substrate; and,

(e) correlating the amount of phosphate transferred to said synthetic peptide substrate to a concentration of DNA-PK activity in said biological sample.

2. The method of Claim 1, further comprising contacting said biological sample with a preparation of double-stranded DNA.

3. The method of Claim 2, wherein said preparation of double-stranded DNA is substantially linear.

4. The method of Claim 2, wherein said preparation of DNA is provided at a concentration of DNA ranging from about 1 $\mu\text{g/ml}$ to about 1000 $\mu\text{g/ml}$ of said reaction mixture.

5. The method of Claim 2, wherein said preparation of DNA is provided at a concentration of DNA ranging from about 5 $\mu\text{g/ml}$ to about 15 $\mu\text{g/ml}$ of said reaction mixture.

6. The method of Claim 2, wherein said preparation of DNA is provided at a concentration of DNA of about 10 $\mu\text{g/ml}$ of said reaction mixture.

7. The method of Claim 1, wherein said detectably-labeled phosphate donor is selected from the group consisting of gamma labeled [^{32}P]-ATP, [^{32}P]-dATP, [^{33}P]-ATP or [^{33}P]-dATP and mixtures thereof.

004207 2E456960

10

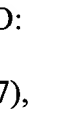
no enhancer
aa.

TO: *rw*

7),

1

ro



2 phosphorylation sites

←

13. The method of Claim 1, wherein said synthetic peptide substrate is selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Asn Asn Val Leu Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Lys Lys (SEQ ID NO: 6), Met Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg (SEQ ID NO: 7), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Leu Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 14), Glu Pro Pro Gln

Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Leu Thr Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 16), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

14. The method of Claim 1, wherein said synthetic peptide substrate is identical to or a variant of the amino acid sequences found at the amino terminus of human or murine p53 tumor suppressor proteins and which contains Ser¹⁵ of human p53 tumor suppressor protein or Ser⁷ or Ser¹⁸ of murine p53 tumor suppressor protein.

15. The method of Claim 14, wherein said synthetic peptide substrate is selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18), Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19), Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys

Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 63), and Pro Leu Ser Gln Glu Ala Phe Ala Gly
Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 64).

16. The method of Claim 15, wherein said synthetic peptide substrate is
selected from the group consisting of Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu
Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp
Lys Lys (SEQ ID NO: 15), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys
(SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID
NO: 19).

17. The method of Claim 1, wherein said incubating time ranges from about 1
second to about 60 minutes.

18. The method of Claim 17, wherein said incubating time ranges from about
2 minutes to about 15 minutes.

19. The method of Claim 18, wherein said incubating time ranges from about
7 minutes to about 10 minutes.

20. The method of Claim 1, wherein said incubating is at a temperature
ranging from about 5°C to about 45°C.

21. The method of Claim 20, wherein said incubating is at a temperature
ranging from about 20°C to about 37°C.

22. The method of Claim 21, wherein said incubating is at a temperature of
about 30°C.

23. A composition useful for detecting and quantitating DNA-activated
protein kinase (DNA-PK) activity in a biological sample, comprising a synthetic peptide
substrate defined by the following features to provide specific recognition and

004207-102400

phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor.

24. The composition of Claim 23, wherein said first spacer sequence comprises 1 to 4 amino acids.

25. The composition of Claim 24, wherein said first spacer sequence comprises 1 or 2 amino acids.

26. The composition of Claim 23, wherein said second spacer sequence comprises at least seven and no more than twenty-three amino acids.

27. The composition of Claim 26, wherein said second spacer sequence comprises at least seven and no more than eleven amino acids.

28. The composition of Claim 26, wherein said second spacer sequence comprises seven amino acids.

29. The composition of Claim 23, wherein said first and second spacer sequences exclude serine, threonine and tyrosine.

004207-2E156960

30. The composition of Claim 23, wherein said synthetic peptide substrate is selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Asn Asn Val Leu Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Lys Lys (SEQ ID NO: 6), Met Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg (SEQ ID NO: 7), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Leu Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 14), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Leu Thr Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 16), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

31. The composition of Claim 23, wherein said synthetic peptide substrate is identical to or a variant of the amino acid sequences found at the amino terminus of human or murine p53 tumor suppressor proteins and which contains Ser¹⁵ of human p53 tumor suppressor protein or Ser⁷ or Ser¹⁸ of murine p53 tumor suppressor protein.

32. The composition of Claim 31, wherein said synthetic peptide substrate is selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18), Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19), Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 63), and Pro Leu Ser Gln Glu Ala Phe Ala Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 64).

33. The composition of Claim 32, wherein said synthetic peptide substrate is selected from the group consisting of Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

34. A composition useful for detecting and quantitating DNA-activated protein kinase (DNA-PK) activity in a biological sample, comprising a synthetic peptide substrate selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

35. A composition according to Claim 34, wherein the peptide substrate is selected from the group consisting of Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

36. A kit for detecting and quantitating DNA-activated protein kinase (DNA-PK) activity, comprising:

(a) a detectably-labeled phosphate donor;

(b) a composition useful for specific detection and quantitation of DNA-PK which comprises a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor;

(c) means for detecting a labeled synthetic peptide substrate, whereby detection of labeled synthetic peptide substrate is utilized to determine an amount of DNA-PK activity in said biological sample.

37. The kit of Claim 36, further including double-stranded DNA.
38. The kit of Claim 37, wherein said double-stranded DNA is substantially linear.
39. The kit of Claim 36, wherein said detectably labeled phosphate donor is selected from the group consisting of gamma labeled [^{32}P]-ATP, [^{32}P]-dATP, [^{33}P]-ATP, [^{33}P]-dATP and mixtures thereof.

40. The kit of Claim 39, wherein said detectably labeled phosphate donor is [³²P]-ATP.

41. The kit of Claim 36, wherein said synthetic peptide substrate is selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Asn Asn Val Leu Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Lys Lys (SEQ ID NO: 6), Met Ala Ile Tyr Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg (SEQ ID NO: 7), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Leu Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 14), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Leu Thr Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 16), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

42. The kit of Claim 36, wherein said synthetic peptide substrate is identical to or a variant of the amino acid sequences found at the amino terminus of human or murine

004201-2256960

p53 tumor suppressor proteins and which contains Ser¹⁵ of human p53 tumor suppressor protein or Ser⁷ or Ser¹⁸ of murine p53 tumor suppressor protein.

43. The kit of Claim 42, wherein said synthetic peptide substrate is selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18), Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19), Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 63), and Pro Leu Ser Gln Glu Ala Phe Ala Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 64).

44. The kit of Claim 43, wherein said synthetic peptide substrate is selected from the group consisting of Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

45. The kit of Claim 36, further including a negative control peptide of similar composition to the synthetic peptide substrate which is not phosphorylated by DNA-PK.

46. The kit of Claim 45, wherein said negative control peptide is identical in amino acid composition to said synthetic peptide substrate.

47. The kit of Claim 46, wherein said negative control peptide is selected from the group consisting of Glu Pro Pro Leu Ser Glu Gln Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 20) and Pro Glu Ser Glu Gln Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 23).

48. The kit of Claim 36, wherein said negative control peptide is identical to said synthetic peptide substrate except that the serine or threonine of said amino acid pairs Ser-Gln or Gln-Ser or Thr-Gln or Gln-Thr is replaced with alanine.

49. The kit of Claim 48, wherein said negative control peptide is selected from the group consisting of Glu Pro Pro Leu Ala Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 21), Pro Glu Glu Ala Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 24) and Pro Glu Glu Ser Glu Gln Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 25).

50. The kit of Claim 36, further including buffers.

51. The kit of Claim 36, further including a preparation of DNA-PK.

52. The kit of Claim 36, further including a reagent to detect a labeled synthetic peptide substrate.

53. A method for detecting the presence of linear double-stranded DNA in a biological sample, comprising the steps of:

004201-26456960

- 00420T 22456960
06643 103400
- 10
- (a) forming a reaction mixture by contacting a biological sample with a detectably-labeled phosphate donor, a preparation of DNA-PK and a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor;
- 20
- (b) incubating said reaction mixture for a time and at a temperature to allow the transfer of phosphate from said phosphate donor to said synthetic peptide;
- (c) stopping the transfer of phosphate from said phosphate donor to said synthetic peptide;
- (d) determining the amount of phosphate transferred from said phosphate donor to said synthetic peptide; and
- (e) correlating the amount of phosphate transferred to said synthetic peptide to a concentration of DNA in said biological sample.

54. The method of Claim 53, wherein said biological sample is contacted with said detectably-labeled phosphate donor selected from the group consisting of gamma labeled [^{32}P]-ATP, [^{32}P]-dATP, [^{33}P]-ATP or [^{33}P]-dATP and mixtures thereof.

55. The method of Claim 54, wherein said detectably-labeled phosphate donor is provided at a concentration ranging from about 20 μM to about 1 mM.

56. The method of Claim 53, wherein said incubating time ranges from about 1 second to about 60 minutes.

57. The method of Claim 53, wherein said incubating is at a temperature from about 5°C to about 45°C.

58. The method of Claim 53, wherein said preparation of DNA-PK is added to provide a concentration of DNA-PK ranging from about 0.1 U/ml to about 10 U/ml.

59. The method of Claim 53, wherein said synthetic peptide substrate is added to provide a concentration of substrate ranging from about 50 μM to about 1 mM.

60. The method of Claim 53, wherein said biological sample is contacted with said synthetic peptide substrate selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Asn Asn Val Leu Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Lys Lys (SEQ ID NO: 6), Met Ala Ile Try Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg (SEQ ID NO: 7), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys

004207-2E456960

Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Leu Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 14), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Leu Thr Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 16), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

61. The method of Claim 53, wherein said biological sample is contacted with said synthetic peptide substrate which is identical to or a variant of the amino acid sequences found at the amino terminus of human or murine p53 tumor suppressor proteins and which contains Ser¹⁵ of human p53 tumor suppressor protein or Ser⁷ or Ser¹⁸ of murine p53 tumor suppressor protein.

62. The method of Claim 61, wherein said biological sample is contacted with a synthetic peptide substrate selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO:

004201-2656960

12), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18), Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19), Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 63), and Pro Leu Ser Gln Glu Ala Phe Ala Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 64).

63. The method of Claim 62, wherein said biological sample is contacted with a synthetic peptide substrate selected from the group consisting of Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

64. A method for detecting the presence in a sample of a substance that alters the activity of DNA-PK, comprising the steps of:

(a) forming a series of reaction mixtures in which increasing amounts of said sample are contacted with a detectably-labeled phosphate donor, a preparation of DNA-PK, a preparation of linear double-stranded DNA and a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence

004207-2345960

at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor;

(b) incubating said reaction mixtures for a time and at a temperature to allow the transfer of phosphate from said phosphate donor to said synthetic peptide;

(c) stopping the transfer of phosphate from said phosphate donor to said synthetic peptide;

(d) determining the amount of phosphate transferred from said phosphate donor to said synthetic peptide in each reaction mixture of said series; and

(e) correlating the amount of phosphate transferred to said synthetic peptide in each reaction mixture of said series with the presence of a DNA-PK activity altering substance in said sample.

65. The method of Claim 64, wherein said biological sample is contacted with said detectably-labeled phosphate donor selected from the group consisting of gamma labeled [^{32}P]-ATP, [^{32}P]-dATP, [^{33}P]-ATP or [^{33}P]-dATP and mixtures thereof.

66. The method of Claim 65, wherein said detectably-labeled phosphate donor is provided at a concentration ranging from about 20 μM to about 1 mM.

67. The method of Claim 64, wherein said incubating time ranges from about 1 second to about 60 minutes.

004207-26456960

68. The method of Claim 64, wherein said incubating is at a temperature from 5°C to about 45°C.

69. The method of Claim 64, wherein said preparation of DNA-PK is added to a concentration of DNA-PK ranging from about 0.1 U/ml to about 10 U/ml.

70. The method of Claim 64, wherein said synthetic peptide substrate is added
 71. at a concentration of substrate ranging from about 50 μ M to about 1 mM.

71. The method of Claim 64, wherein said synthetic peptide substrate is
d from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro
u Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1),
u Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly
p Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu
u Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val
o Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Asn
al Leu Ser Pro Leu Pro Ser Gln Ala Met Asp Asp Leu Met Lys Lys (SEQ ID NO:
t Ala Ile Try Lys Gln Ser Gln His Met Thr Glu Val Val Arg Arg (SEQ ID NO: 7),
o Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu
o Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro
u Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro
r Leu Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 14), Glu Pro Pro Gln
n Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Leu Thr
u Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 16), Glu Pro Pro Asp Ser Gln
a Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala

Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

72. The method of Claim 64, wherein said biological sample is contacted with said synthetic peptide substrate which is identical to or a variant of the amino acid sequences found at the amino terminus of human or murine p53 tumor suppressor proteins and which contains Ser¹⁵ of human p53 tumor suppressor protein or Ser⁷ or Ser¹⁸ of murine p53 tumor suppressor protein.

73. The method of Claim 72, wherein said biological sample is contacted with a synthetic peptide substrate selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Glu Pro Pro Leu Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Asp Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18), Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19), Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 63), and Pro Leu Ser Gln Glu Ala Phe Ala Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 64).

004207-2255960

second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor;

(b) incubating said reaction mixture for a time and at a temperature to allow the cleavage of phosphate from said synthetic peptide;

(c) stopping the cleavage of phosphate from said synthetic peptide;

(d) determining the amount of phosphate cleaved from said synthetic peptide; and

(e) correlating the amount of phosphate cleaved from said synthetic peptide to the presence of protein phosphatase in said biological sample.

79. The method of Claim 78, wherein said biological sample is contacted with said synthetic peptide substrate selected from the group consisting of Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu PO₄-Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Leu Leu Pro Glu (SEQ ID NO: 1), Met Glu Glu PO₄-Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Leu Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro (SEQ ID NO: 2), Met Glu Glu PO₄-Ser Gln Ser Asp Ile Ser Leu Glu Leu Pro Tyr Lys Lys (SEQ ID NO: 3), Met Glu Glu Pro Gln Ser Asp Pro Ser Val Glu Pro Pro Leu PO₄-Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 4), Glu Pro Pro Leu PO₄-Ser Gln Glu Thr Phe Ser Asp Leu Trp Lys Lys (SEQ ID NO: 8), Glu Pro Pro Leu PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Leu PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Leu Lys Lys (SEQ ID NO: 12), Glu Pro

Pro Gln PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Glu Pro Pro Asp PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 17), Pro Glu Glu PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18), Pro Glu PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19), Pro Leu PO₄-Ser Gln Glu Thr Phe Ser Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 63), and Pro Leu PO₄-Ser Gln Glu Ala Phe Ala Gly Leu Trp Lys Leu Leu Pro Pro-Lys Lys (SEQ ID NO: 64).

80. The method of Claim 79, wherein said biological sample is contacted with a synthetic peptide substrate selected from the group consisting of Glu Pro Pro Leu PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Pro Glu Glu PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu PO₄-Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

81. The method of Claim 78, wherein said synthetic peptide substrate is added to provide a concentration of substrate ranging from about 50 μ M to about 1 mM of said reaction mixture.

82. The method of Claim 78, wherein said incubating time ranges from about 1 second to about 60 minutes.

83. The method of Claim 78, wherein said incubating temperature ranges from about 5°C to about 45°C.

84. A method for monitoring protein kinase activity in a cell, comprising:
(a) introducing an expression vector into a cell, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein

segment containing a phosphorylation site consensus sequence motif specific for said protein kinase, an optional nuclear localization signal, an optional DNA-binding domain, and a detectable epitope;

(b) expressing said protein substrate to provide a phosphorylatable protein substrate;

(c) determining the amount of phosphorylated protein substrate; and

(d) correlating the amount of phosphorylated protein substrate to a concentration of said protein kinase activity in said cell.

85. A method for monitoring DNA-activated protein kinase (DNA-PK) activity in a cell, comprising:

(a) introducing an expression vector into a cell, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for DNA-PK, a nuclear localization signal, a DNA-binding domain, and a detectable epitope;

(b) expressing said protein substrate to provide a phosphorylatable protein substrate;

(c) determining the amount of phosphorylated protein substrate; and

(d) correlating the amount of phosphorylated protein substrate to a concentration of DNA-PK activity in said cell.

86. The method of Claim 84, wherein said protein substrate further comprises:

(a) an epitope for affinity purification ; and

(b) a cleavage site, said cleavage site permitting excision of said phosphorylation site from said protein segment.

87. The method of Claim 85, wherein said protein substrate further comprises:

- (a) an epitope for affinity purification; and
- (b) a cleavage site, said cleavage site permitting excision of said phosphorylation site from said protein segment.

88. A composition for monitoring protein kinase activity in a cell, comprising an expression vector, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for said protein kinase, an optional nuclear localization signal, an optional DNA-binding domain, and a detectable epitope.

89. A composition for monitoring DNA-activated protein kinase (DNA-PK) activity in a cell, comprising an expression vector, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for DNA-PK, a nuclear localization signal, a DNA-binding domain, and a detectable epitope.

90. The composition of Claim 89, wherein said expression vector is substantially identical to p422SUB1.

91. A kit for monitoring protein kinase activity in a cell, comprising:

- (a) an expression vector, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a

09595437-102400

phosphorylation site consensus sequence motif specific for said protein kinase, an optional nuclear localization signal, an optional DNA-binding domain, and a detectable epitope;

(b) means for detecting a phosphorylated protein substrate, whereby detection of said phosphorylated protein substrate is utilized to determine an amount of said protein kinase activity in said cell.

92. A kit for monitoring DNA-activated protein kinase (DNA-PK) activity in a cell, comprising:

(a) an expression vector, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for DNA-PK, a nuclear localization signal, a DNA-binding domain, and a detectable epitope;

(b) means for detecting a phosphorylated protein substrate, whereby detection of said phosphorylated protein substrate is utilized to determine an amount of DNA-PK activity in said cell.

93. A method for identifying agents that are able to alter the activity of a protein kinase in a cell, comprising:

(a) introducing an expression vector into a first and a second population of cells, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for said protein kinase, an optional nuclear localization signal, an optional DNA-binding domain, and a detectable epitope;

(b) contacting said first population of cells with said agent;

09695437-102400

(c) expressing said protein substrate in each population of cells to provide a phosphorylatable protein substrate;

(d) determining an amount of phosphorylated protein substrate in each population of cells;

(e) comparing the amount of phosphorylated protein determined in step (d) to establish a difference in the amount of phosphorylated protein; and

(f) correlating said difference in the amount of phosphorylated protein in the two populations to the ability of said agent to alter the activity of said protein kinase in a cell.

94. A method for identifying agents that are able to alter the activity of DNA-activated protein kinase (DNA-PK) in a cell, comprising:

(a) introducing an expression vector into a first and a second populations of cells, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for DNA-PK, a nuclear localization signal, a DNA-binding domain, and a detectable epitope;

(b) contacting said first population of cells with said agent;

(c) expressing said protein substrate in each population of cells to provide a phosphorylatable protein substrate;

(d) determining an amount of phosphorylated protein substrate in each population of cells;

(e) comparing the amount of phosphorylated protein determined in step (d) to establish a difference in the amount of phosphorylated protein; and

10

10

09695437-102400

(f) correlating said difference in the amount of phosphorylated protein in the two populations to the ability of said agent to alter the activity of DNA-PK in a cell.

95. A method for monitoring a protein kinase activity in a cell, comprising:

(a) introducing an expression vector into a cell, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for said protein kinase, an optional nuclear localization signal, an optional DNA-binding domain, and a detectable epitope;

(b) expressing said protein substrate to provide a phosphorylatable protein substrate, said phosphorylatable protein substrate being capable of activating expression of a reporter gene to provide a reporter gene product;

(c) determining an amount of reporter gene product; and

(d) correlating the amount of reporter gene product to a concentration of said protein kinase activity in said cell.

96. A method for monitoring DNA-activated protein kinase (DNA-PK) activity in a cell, comprising:

(a) introducing an expression vector into a cell, said expression vector containing a gene coding for a protein substrate, said protein substrate having a protein segment containing a phosphorylation site consensus sequence motif specific for DNA-PK, a nuclear localization signal, a DNA-binding domain, and a detectable epitope;

(b) expressing said protein substrate to provide a phosphorylatable protein substrate, said phosphorylatable protein substrate being capable of activating expression of a reporter gene to provide a reporter gene product;

004207-2356960

10

- (c) determining an amount of reporter gene product; and
- (d) correlating the amount of reporter gene product to a concentration of DNA-PK activity in said cell.

97. A method for detecting and quantitating DNA-activated protein kinase (DNA-PK) activity in a biological sample, comprising the steps of:

- (a) forming a reaction mixture by contacting a biological sample with a phosphate donor and a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor in the reaction mixture;
- (b) incubating said reaction mixture for a time and at a temperature to allow the transfer of phosphate from said phosphate donor to said synthetic peptide substrate;
- (c) stopping the transfer of phosphate from said phosphate donor to said synthetic peptide substrate;

20

004201010640009695437

- (d) separating phosphorylated peptide substrates from said peptide substrate to which no phosphate was transferred;
 - (e) determining an amount of phosphorylated synthetic peptide substrate;
- and,
- (f) correlating said amount of phosphorylated synthetic peptide substrate to a concentration of DNA-PK activity in said biological sample.

98. The method of Claim 98 in which the phosphate donor is ATP.

99. A kit for detecting and quantitating DNA-activated protein kinase (DNA-PK) activity, comprising:

- (a) a phosphate donor;
- (b) a composition useful for specific detection and quantitation of DNA-PK which comprises a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor;

004207 245960

(c) means for detecting a phosphorylated synthetic peptide substrate, whereby detection of said phosphorylated synthetic peptide substrate is utilized to determine an amount of DNA-PK activity in said biological sample.

100. The kit of Claim 99, wherein said phosphate donor is ATP.

101. The kit of Claim 99, wherein said synthetic peptide substrate is selected from the group consisting of Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 15), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys Lys (SEQ ID NO: 19).

102. The kit of Claim 99, further including a negative control peptide of similar composition to the synthetic peptide substrate which is not phosphorylated by DNA-PK.

103. The kit of Claim 99, further including buffers.

104. The kit of Claim 99, further including a preparation of DNA-PK.

105. The kit of Claim 99, further including a reagent to detect a phosphorylated peptide substrate.

106. A method for detecting the presence in a sample of a substance that alters the activity of DNA-PK, comprising the steps of:

(a) forming a series of reaction mixtures in which increasing amounts of said sample are contacted with a phosphate donor, a preparation of DNA-PK, a preparation of linear double-stranded DNA and a synthetic peptide substrate defined by the following features to provide specific recognition and phosphorylation by DNA-PK: (1) a

004207 103400 066543 245960

phosphate-accepting amino acid pair which may include serine-glutamine (Ser-Gln) (SQ), threonine-glutamine (Thr-Gln) (TQ), glutamine-serine (Gln-Ser) (QS), or glutamine-threonine (Gln-Thr) (QT); (2) enhancer amino acids which may include glutamic acid or glutamine immediately adjacent at the amino- or carboxyl- side of the amino acid pair and forming an amino acid pair-enhancer unit; (3) a first spacer sequence at the amino terminus of the amino acid pair-enhancer unit; (4) a second spacer sequence at the carboxyl terminus of the amino acid pair-enhancer unit, which spacer sequences may include any combination of amino acids that does not provide a phosphorylation site consensus sequence motif; and (5) a tag moiety, which may be an amino acid sequence or another chemical entity that permits separating the synthetic peptide from the phosphate donor;

(b) incubating said reaction mixtures for a time and at a temperature to allow the transfer of phosphate from said phosphate donor to said synthetic peptide;

(c) stopping the transfer of phosphate from said phosphate donor to said synthetic peptide;

(d) determining an amount of phosphorylated synthetic peptide in each reaction mixture of said series; and

(e) correlating said amount of phosphorylated synthetic peptide in each reaction mixture of said series with the presence of a DNA-PK activity altering substance in said sample.

107. The method of Claim 106, wherein said phosphate donor is ATP.

108. The method of Claim 106, wherein said synthetic peptide substrate is selected from the group consisting of Glu Pro Pro Leu Ser Gln Glu Ala Phe Ala Asp

004202 2E45960

Leu Trp Lys Lys (SEQ ID NO: 11), Glu Pro Pro Gln Ser Gln Glu Ala Phe Ala Asp
Leu Trp Lys Lys (SEQ ID NO: 15), Pro Glu Glu Ser Gln Glu Ala Phe Ala Asp Leu
Trp Lys Lys (SEQ ID NO: 18) and Pro Glu Ser Gln Glu Ala Phe Ala Asp Leu Trp Lys
Lys (SEQ ID NO: 19).

109. The method of Claim 106, wherein said increasing amounts of sample
range from zero percent, by volume, of a reaction mixture to about fifty percent, by
10 volume, of a reaction mixture.

110. The method of Claim 106, wherein said sample comprises a biological
sample.

111. The method of Claim 106, wherein said sample comprises a chemical
solution.

004207-2E456960